Degradation of phytic acid during soybean seedling growth

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《Original Articles》

Degradation of phytic acid during soybean seedling growth

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Abstract

Phytic acid is stored as a source of phosphorus in the seeds of plants. When monogastric animals consume seeds or foods derived from seeds, phytic acid inhibits the absorption of minerals and blocks the activity of digestive proteases. Therefore, phytic acid should be removed from seeds or seed derivatives. In this study, we analyzed the amount of phytic acid in soybeans at various stages of growth under dark conditions. We found that phytic acid levels were reduced beginning at day 4. By day 6, while the majority of seed protein remained, no phytic acid could be detected. Therefore, our data suggested that soybeans grown for several days after germination were high in nutritional value, with low phytic acid content.

Keywords: phytic acid; phytate; seed; germination; soybean

Introduction

During seed maturation in higher plants, phytic acid (*myo*-inositol-6-phosphate) is synthesized by the condensation of *myo*-inositol-1-phosphate synthesized from glucose 6-phosphate and five phosphate molecules provided by adenosine triphosphate (ATP) hydrolysis (1). Phytic acid then accumulates in the protein body (2). Phytic acid is found in cereal grains, legume seeds, and oilseeds and accounts for 1%-8% of the dry weights of these materials (3). About 90% of phosphorus in seeds is bound to phytic acid, and this source of phosphate is essential for nucleotide and phospholipid synthesis as immature roots of younger individuals are not able to take up sufficient inorganic phosphate from the soil. Moreover, phytic acid strongly chelates various metal ions and is therefore involved in the preservation and detoxification of metals. During germination, phytic acid is broken down by phytase, releasing phosphorus, metal ions, and *myo*-inositol for use by the growing individual (4). However, the precise timing and rate of the decrease in phytic acid is not yet known.

Monogastric animals lack phytase for hydrolysis of phytic acid in the digestive tract. Therefore, intake of cereal grains or legumes containing phytic acid can inhibit the absorption of metal ions, such as Zn^{2+}, Mg^{2+}, Fe^{3+}, and Ca^{2+}, by monogastric animals (5). Additionally, phytic acid interacts with and inhibits the activity of digestive proteases (6, 7). Therefore, researchers have been attempting to develop methods for breeding plants with less phytic acid; however, most modified plants showing low phytic acid content also have defects, such as reduced seed weight, failure to germinate, and stunted growth (8).

Seeds of soybeans (*Glycine Max* [L.] Merrill),

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which are native to eastern Asia, are used for production of various traditional foods, such as soybean curds and soy source. Soybean seeds contain proteins and lipids that are rich in essential amino acids and essential fatty acids (9, 10). Moreover, soybean seeds contain other components, such as isoflavone and saponin, which have antioxidative, anticancer, and antiviral effects, and they have been shown to lower blood lipid levels and blood pressure (11, 12). Therefore, soybean seeds are considered a healthful food and are thought to help reduce diseases in adults. Proteins isolated from soybean seeds also have several useful properties, such as water retention, heat-induced gelation, and emulsifiability, making soybean seeds useful in the production of various processed foods (13).

Despite these healthful properties, soybean seeds contain phytic acid at concentrations of up to 2%-3% dry weight (14). In addition, many food products produced from soybeans, such as soybean milk and flour, contain phytic acid, and the isolated soy protein used to produce many processed foods also contains nearly as much phytic acid as is found in seeds because phytic acid can bind to protein (15). Moreover, phytic acid has been shown to influence the quality of soybean curd by chelating coagulants (14).

Based on these previous works, it is clear that methods for removing phytic acid from soybeans would be beneficial in the production of soybean-based products. Therefore, in this study, we investigated the changes in phytic acid levels during the germination and growth of soybeans. The results from our research provide insights into the levels of phytic acid in soybeans at different times during growth and may contribute to the development of simple cultivation strategies for reducing levels of phytic acid in soybean-based products.

Results and Discussion

Changes in dry or fresh weight of soybeans during germination and growth

To calculate phytic acid contents, we measured dry and fresh weights of soybean plants at various times during growth for individuals grown under dark conditions. The dry weights of individual plants remained constant throughout the entire growth period in this experiment (Figure 1). These data indicated that the total non-water components of soybeans (e.g., sugars, proteins, lipids, and others) did not increase under our growth conditions, likely due to the lack of supplementation with elements such as nitrogen, phosphorus, and potassium, which are needed for normal growth and *de novo* synthesis of many substances. In addition, since photosynthesis was not occurring in the dark conditions used in this study, there was not much energy available for growth and synthesis.

Consistent with this, no increases in fresh weights were observed until 1 day after sowing, as was observed for dry weights (Figure 1). However, after this point, the fresh weights of plants began to increase. This was consistent with the completion of germination (root emergence), after which water absorption begins. Therefore, at day 1 after sowing, soybeans were assumed to have almost finished germination in our experiment.

The fresh weights of individual plants continued to increase until the end of the growth period (Figure 1), indicating that germinated individuals, known as seedlings, were absorbing water. Thus, cell expansion and tissue elongation occurred despite the lack of elements or energy for synthesis of substances.

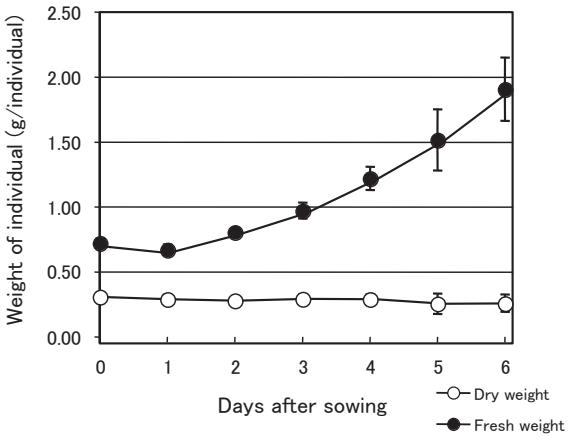

Figure 1. Changes in weights of soybean plants during germination and growth under dark conditions. Open circles indicate mean dry weights of three plants desiccated at 60°C for 4 days. Closed circles indicate mean fresh weights of three plants. Error bars represent standard deviations.

Changes in protein levels in soybeans during germination and growth

To compare the amounts of phytic acid according to the levels of protein, we determined the protein levels in soybean plants grown under dark conditions. There were no differences in protein levels between seeds at day 0 and germinated seeds at day 2 (Figure 2). However, in 4-day-old seedlings, protein levels were slightly lower than those in 2-day-old germinated seeds. Moreover, protein amounts were further decreased in 6-day-old seedlings (74% that of seeds at day 0). These results suggested that protein started to degrade after germination was completed.

Most seed protein is thought to be stored during seed maturation as a source of amino acids and nitrogen (16). However, some enzymes and transporters involved in the process of germination are also stored in seeds. Therefore, there is not much need for additional amino acids until after germination, when cell division, tissue expansion, and plant metabolism are initiated. At that time, huge amounts of amino acids are needed for *de novo* protein and nucleotide synthesis. At the same time, much of the seed protein is degraded. This is consistent with our results showing that protein levels were decreased following the completion of germination.

Changes in phytic acid levels in soybeans during germination and growth

The complex of Fe^{3+} and sulfosalicylic acid is called Wade reagent and exhibits a deep purple color, which is altered by addition of phytic acid (17). Therefore, we used Wade reagent to measure changes in phytic acid concentrations in soybean seeds and seedlings during germination and growth. We created a standard curve for phytic acid amounts ranging from 0 to 1 mg and obtained a regression line (Figure 3), which was subsequently used for quantification of phytic acid contained in soybean plants.

To elucidate the time-dependent changes in phytic acid levels, we measured phytic acid amounts in soybean seeds, germinated seeds, and seedlings (Figure 4). No differences were observed between

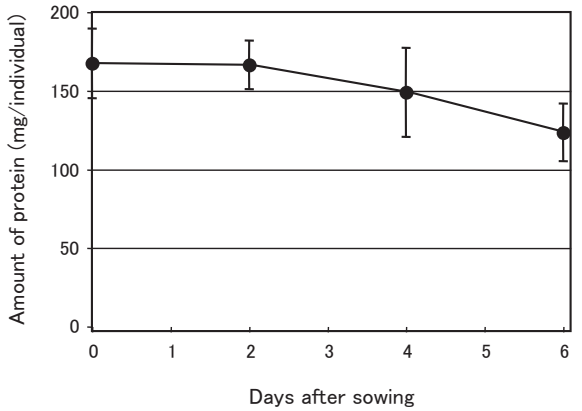

Figure 2. Changes in protein levels in soybean plants during germination and growth. Closed circles indicate mean protein content from three plants grown under dark conditions. Error bars represent standard deviations.

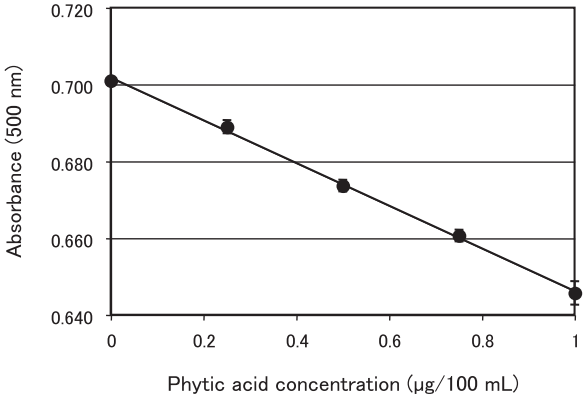

Figure 3. Standard curve for the phytic acid assay. Closed circles represent mean absorbance at 500 nm of mixed solutions of Wade reagent and phytic acid at known concentrations. Absorbances of three individual mixtures were averaged. Error bars represent standard deviations. The line represents the regression line. The squared coefficient of correlation was 0.999. When the phytic acid concentration is x and absorbance (at 500 nm) is y, their relationship is represented by following linear equation: $y = 0.0556x + 0.702$.

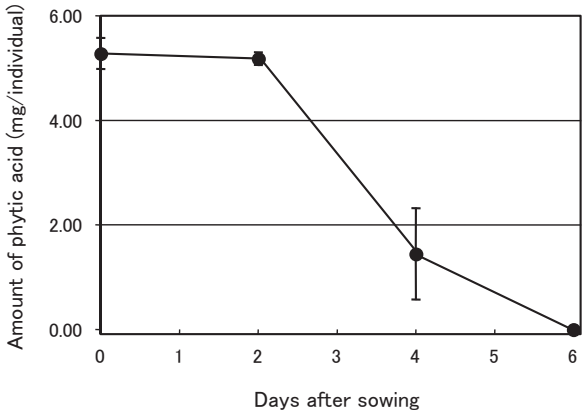

Figure 4. Changes in the amounts of phytic acid in soybean plants during germination and growth. Closed circles indicate mean phytic acid content measured using three plants grown under dark conditions. Error bars represent standard deviations.

	Phytic acid contents per d.w. (%) (± SD)	Phytic acid contents per f.w. (%) (± SD)	Ratios of phytic acid to protein (mg/mg) (± SD)
0 day	1.70 ± 0.200	0.731 ± 0.0557	0.0314 ± 0.00453
2 days	1.85± 0.121	0.643 ± 0.0246	0.0310 ± 0.00300
4 days	0.499 ± 0.310	0.119 ± 0.0722	0.00973 ± 0.00616

Table 1. Phytic acid contents and ratios of phytic acid to protein in soybean plants at 0, 2, and 4 days after sowing. d.w. and f.w. represent dry weight and fresh weight of individual plants. Ratios of phytic acid to protein were calculated by phytic acid amounts (mg) divided by protein amounts (mg). Each standard deviation was calculated by the coefficients of variation for phytic acid amounts, dry weights, fresh weights, or protein amounts.

seeds at day 0 and germinated seeds at day 2. However, phytic acid levels were dramatically reduced in 4-day-old seedlings (27% that of 0-day-old seeds) and were not detected in 6-day-old seedlings. These results suggested that phytic acid degradation began after germination.

During seed maturation, phytic acid is stored as a source of phosphorus, which is required for the synthesis of nucleotides and phospholipids. However, ribonucleic acids (RNA) encoding the protein required for germination or processes that occur immediately after germination are synthesized during seed maturation and stored in mature seeds. In addition, cell division and tissue expansion, which require phospholipid synthesis, are initiated after the completion of germination. Therefore, there is not much need for additional phosphorus until after germination. These observations support our results demonstrating that phytic acid levels decreased after germination.

Table 1 shows phytic acid contents expressed as a percentage of soybean dry weight. Similar to our results in the experiments described above, there were no differences in phytic acid contents between seeds at day 0 and germinated seeds at day 2. However, the phytic acid content of 4-day-old seedlings was about 29% that of 0-day-old germinated seeds. These data suggest that phytic acid was degraded more rapidly than other substances in order to release phosphorus for seedling growth after germination has finished.

In contrast, when expressed as a percentage of soybean fresh weight, phytic acid levels in 4-day-old seedlings were only about 16% that of day 0 seeds (Table 1). This result was expected because the fresh weight of 4-day-old seedlings was about twice that of day 0 seeds. These results based on the fresh weight of soybeans indicated that phytic acid intake could be minimized by consumption or use of soybean seeds at 4 days after sowing.

To assess the quality of soy protein in plants with low phytic acid content, we calculated the ratio of phytic acid content to protein content in individual plants (Table 1). There were no differences in ratios of phytic acid to protein between day 0 seeds and 2-day-old germinated seeds. However, the ratio of phytic acid to protein in 4-day-old seedlings was only about one-third that of day 0 seeds. These data demonstrated that phytic acid was degraded more rapidly than protein during seed germination and that 4-day-old seedlings could be used to produce soy protein containing low levels of phytic acid.

While 6-day-old seedlings contained much less phytic acid (indeed, phytic acid could not even be detected in 6-day-old seedlings in our analysis), the extended growth period would create substantially increased costs. Therefore, we believe that reducing phytic acid to the levels in 4-day-old seedlings would be practical for the purposes of soybean-based food production.

Conclusion

Because soy protein has several advantages (e.g., amino acid composition, processing properties, and applications as a functional food), isolated soy protein is used to produce various processed foods or functional foods. Our results showed that it was possible to reduce or eliminate phytic acid levels while maintaining high protein levels. Therefore, we propose that soy protein isolated from seedlings several days after germination should be used to produce various foods. Because these foods would have reduced phytic acid content, they would show higher nutrient content than conventional soybean-based processed foods.

In imbibing seeds, germination is accompanied by the repair of membranes and organelles and only minimal protein synthesis; however, for cell division, tissue expansion, photosynthesis, and absorption of elements from soil, germinated seeds must synthesize a variety of components. These ideas are consistent with our result demonstrating that degradation of protein and phytic acid did not start until germination had finished.

Moreover, phytic acid degradation appeared to be more rapid than protein degradation, indicating a greater need for phosphorus than amino acids during seedling growth (i.e., for production of nucleotides and phospholipids). Therefore, taken together, our data suggested that cell division and tissue expansion preceded photosynthesis and other metabolic activities. Because the soybeans used in our experiment were grown under dark conditions, seedlings had to elongate to obtain light. Thus, in future experiments, we can explore conditions (e.g., temperature, soil moisture content, and fertilizer) that promote phosphorus utilization and may lead to even more rapid degradation of phytic acid.

Materials and Methods

Plant materials

For growth of soybean plants, seeds of *Glycine Max* (L.) Merrill (ecotype Enrei) were sown in pods filled with expanded vermiculite. Watered pods were kept at 25°C for 1–6 days. Day 0 imbibing seeds were prepared by keeping seeds in water at 4°C overnight.

Weight of seedlings

Fresh weight was measured immediately after collection of soy seedlings. Dry weights were measured after desiccation in an incubator at 60°C for 4 days.

Protein concentrations

Extracts for measuring protein contents were prepared from soybean plants homogenized with solution containing 2% sodium dodecyl sulfate and 6% 2-mercaptoethanol. Protein concentrations were assayed according to the Lowry method (18).

Phytic acid levels

Extracts prepared by homogenizing soybean individuals with distilled water were used for quantification of phytic acid levels. The assay was performed according to the methods described by Latta and Eskin (17). Three milliliters of Wade reagent (0.03% solution of $FeCl_3 \cdot 6H_2O$ containing 0.3% sulfosalicylic acid) was added to 9 mL of the extract. The absorbance at 500 nm was measured. The phytic acid concentration of extract was calculated from a standard curve assayed using phytic acid purified from rice (Sigma).

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《原著》

ダイズの発芽に伴うフィチン酸の分解

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要旨

植物種子には、リン酸源としてフィチン酸が貯蔵されている。フィチン酸分解酵素を持たない単胃動物においては、そのフィチン酸がミネラルの吸収や消化酵素の活性を阻害する。したがってフィチン酸は、種子もしくは種子に由来する食品から取り除くべき物質であると考えられている。本研究において我々は、暗下にて様々な期間生育させたダイズ個体に含まれるフィチン酸を定量した。その結果、フィチン酸はダイズの播種後４日目には減少しており、６日目のダイズ個体からは検出されないことが明らかになった。種子貯蔵タンパク質の大部分は播種後６日目のダイズ個体においても残存していたことから、発芽後数日間経過したダイズ個体はフィチン酸含量の少ないタンパク質素材となりうることが示唆された。

キーワード：フィチン酸、種子、発芽、ダイズ、大豆タンパク質

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